The SEISICAT study: a pilot study assessing efficacy and safety of spironolactone in cats with congestive heart failure secondary to cardiomyopathy

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Running Head: Spironolactone use in cats with cardiac failure

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Abstract

Introduction

The pathophysiology of heart failure involves activation of several neurohormonal systems including the renin-angiotensin-aldosterone system. The mineralocorticoid receptor antagonist spironolactone has been shown to be beneficial in humans and dogs with heart failure.

The objective of this pilot study was to investigate the efficacy and safety of spironolactone in cats with heart failure secondary to cardiomyopathy already treated with furosemide and an angiotensin converting enzyme inhibitor.

Animals

Twenty cats with heart failure due to cardiomyopathy.

Methods

The study was a double blind, randomised, placebo-controlled, multicentre clinical study assessing the effect of spironolactone on survival and clinical parameters in cats with heart failure due to cardiomyopathy. The primary endpoint was mortality, defined as death (spontaneous or by euthanasia) due to cardiac causes.

Results

Twenty cats were enrolled: 9 in the spironolactone group and 11 in the placebo group of which 56% (5/9) and 0% (0/11) completed the 15-month period respectively. At inclusion, differences in systemic blood pressure, body condition score, electrocardiographic abnormalities and LA/Ao ratio suggested disease may be less severe in the spironolactone group. Twenty-two percent (2/9) of cats in the spironolactone group and 82% (9/11) in the control group reached the primary endpoint (Fisher's exact test, $p = 0.0216$). No safety issues were identified in either

group.

Conclusions

This study suggests that spironolactone is well-tolerated and preliminary results support further investigation to evaluate the efficacy of spironolactone in the treatment of cats with cardiac failure due to cardiomyopathy.

Key words: aldosterone; feline; mineralocorticoid receptor

Abbreviations

ACEi	angiotensin converting enzyme inhibitor
Ao	aorta
CI	confidence interval
HCM	hypertrophic cardiomyopathy
HR	hazard ratio
LA	left atrium
RAAS	renin angiotensin aldosterone system
SD	standard deviation

Introduction

Cardiomyopathy is the most common form of heart disease and cause of heart failure in cats [1]. The pathophysiology of heart failure involves activation of several neurohormonal systems such as the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) to compensate for the decrease in cardiac output. Aldosterone is a steroid hormone with mineralocorticoid activity and its major physiological function is maintaining sodium and potassium balance and blood pressure control. Binding of aldosterone to the mineralocorticoid receptors in the kidneys results in an increase in sodium and water reabsorption and potassium secretion [2]. This phenomenon increases the extra-cellular fluid volume and thus cardiac preload [3], helping maintain cardiac output in heart failure. Mineralocorticoid receptor antagonists counteract the retention of sodium and water, and reduce aldosterone-induced potassium loss [3, 4]. Mineralocorticoid receptors are also found in cardiomyocytes, coronary endothelial and vascular smooth muscle cells, fibroblasts, and inflammatory cells, such as macrophages [3]. Chronic activation of the RAAS is thought to give rise to deleterious effects and worsening of cardiac function, proarrhythmogenic effects, progression of myocardial fibrosis, vascular remodelling and endothelial dysfunction [5]. Cardiac fibrosis has been reported in 53% of cases of feline hypertrophic cardiomyopathy (HCM) [6] and in the myocardial type of feline restrictive cardiomyopathy [6, 7, 8]. Mineralocorticoid receptor antagonists like spironolactone have been shown to inhibit aldosterone-induced myocardial fibrosis [5] and to reduce remodelling of the vascular smooth muscle cells and myocytes [3]. This action may therefore represent an additional benefit to using these agents in feline cardiomyopathy.

Aldosterone receptor blockade has now been shown to be beneficial in humans with heart failure [9, 10, 11] and in dogs with heart failure secondary to mitral valve disease [4]. Although there is little published work regarding the treatment of cats in heart failure, the current recommendations for treatment include the use of diuretics, angiotensin-converting enzyme inhibitors and antiplatelet drugs if antithrombotic prophylaxis is required [12, 13]. One study of spironolactone at 2mg/kg per os twice daily for 4 months in Maine Coon cats affected by HCM conducted by McDonald et al. in 2008 did not show any improvement in the mitral annular velocity or reduction of the left ventricular mass [14], four of the 13 treated cats developed severe ulcerative facial dermatitis. However, this was a short study of only a few months in cats with sub-clinical disease in a related population. The efficacy and safety of spironolactone are poorly documented in cats with naturally occurring heart disease and congestive heart failure and warrant further investigation due to the drug's mode of action, its antifibrotic properties and its efficacy in heart failure in other species.

The objective of this pilot study was to assess the safety and efficacy of spironolactone in cats with congestive heart failure secondary to cardiomyopathy being treated in combination with furosemide and an angiotensin converting enzyme inhibitor (ACEi).

Animals, Materials and Methods

Study design

The study protocol was approved by the Ethics Committee of the University of Nottingham (reference 135 100118) and an Animal Test Certificate was obtained from the UK Veterinary Medicines Directorate permitting the use of spironolactone in a species for which it was not licensed. This pilot study was a double blind, randomised, placebo-controlled, and multicentre clinical study.

Animals

Client-owned cats with suspected heart failure were screened and those diagnosed with heart failure fulfilling the inclusion criteria were asked to participate in the trial at 3 different cardiology speciality referral centres in the United Kingdom. Cases were seen in practice as primary care or referred cases.

Inclusion criteria

Cats were eligible for inclusion provided the owner had completed and signed an informed consent form. To be enrolled in the study, cats, of any age, gender and breed, had to present with congestive heart failure secondary to cardiomyopathy, with presence of appropriate clinical signs and radiographic evidence of pulmonary oedema and/or pleural effusion due to left sided or biventricular congestive heart failure. The cardiomyopathy had to be confirmed by echocardiographic examination by diploma-holding veterinary cardiologists using currently accepted diagnostic criteria [8] to identify left ventricular remodelling, left atrial (LA) enlargement from both the

long (>16.5 mm) and the short axis views with a left atrial (LA)/Aorta (Ao) ratio of > 1.6, and Doppler evidence of diastolic dysfunction.

Exclusion criteria

Cats with hyperthyroidism, hypertension (Doppler systolic blood pressure >180 mmHg), severe renal disease (serum creatinine >250µmol/l or 2.83mg/dl), congenital heart disease, non-cardiac systemic disease which may affect the outcome (e.g. thromboembolic disease or respiratory disease), pregnancy or lactation were excluded. Cats were also ineligible if a dysrhythmia requiring the use of anti-arrhythmic medication was evident or in cases of previous or ongoing treatment with pimobendan, spironolactone or digoxin.

Randomisation

Cats were randomly allocated to two treatment groups. A randomisation list was prepared using 2N Software[c]. Case allocation was stratified according to the presence of hypertrophic cardiomyopathy or not, and then on the need for hospitalisation or not. This led to the creation of four groups each with a respective randomisation list composed of blocks of four.

Study drugs

The animals were randomly allocated to two groups, the treated group received spironolactone tablets[d] and the control group received a placebo identical to spironolactone in appearance and packaging. Cats were administered from half a tablet to two tablets per day, *i.e.* a spironolactone dose between 1.72mg/kg and

3.33mg/kg per day. In cats, spironolactone is generally used at 2 to 4 mg/kg/day [12, 13]. An allometric extrapolation performed to identify a dose for use in cats provided a similar theoretical dose of 3.15 mg/kg/day for cats with a mean body weight of 3.75kg. For ease of dosing and administration by the owners, only complete 10mg tablets or half tablets were administered, resulting in the above mentioned dose (i.e. 1.72mg/kg to 3.33mg/kg per day). Study drugs were administered for sixty weeks, once daily, with food. The tablet could either be mixed with a small amount of food offered prior to the main meal or administered directly by mouth after feeding. The owners were instructed to mark the date and time the medication was given on the tablet packaging to aid with determination of compliance.

Concomitant treatments

The cats of both groups had to be concomitantly treated with a combination of furosemide and ACEi for congestive heart failure (at least from the day of inclusion). Clopidogrel administration was permitted and left to investigator's discretion. However, administration of pimobendan, anti-arrhythmic medication such as diltiazem, digoxin, lidocaine or beta blockers was forbidden as was the administration of aspirin. Cats previously or currently treated with spironolactone were also excluded from the study. Other concomitant medications, therapies or vaccines were allowed as long as investigators judged they did not interfere with the evaluation of the tested product.

Visit schedule

Visits and assessments were carried out in accordance with the schedule detailed in supplemental Table A on-line.

Parameter assessment

The clinical parameters recorded were appetite change, dyspnoea, demeanour change, behaviour change, syncope, ascites and signs of gastro-intestinal disease. Body condition score was assessed for each cat on a five-point scale.

The radiographic parameters evaluated on plain lateral and dorso-ventral thoracic radiographs were the Buchanan vertebral heart score [15], presence or absence of pleural effusion, pulmonary oedema assessed as none, mild or moderate interstitial, localised or generalised alveolar pattern and the presence or absence of pulmonary venous congestion.

Systolic blood pressure was determined non-invasively at each visit, using a Doppler probe and an appropriate sized inflatable cuff according to guidelines established by the International Society of Feline Medicine[e].

The electrocardiographic parameters evaluated were heart rate and rhythm, ventricular and supraventricular arrhythmias (presence or absence) and any other abnormality using limb leads in right lateral or sternal recumbency.

The following echocardiographic parameters were evaluated on two-dimensional and M-mode images: intraventricular septum thickness in diastole and left ventricular free wall thickness in diastole from the right parasternal long axis view and left ventricular internal dimension in diastole and in systole, left ventricular shortening fraction, left ventricular free wall thickness in diastole and in systole, interventricular septum thickness in diastole and in systole were evaluated with M-mode from a right parasternal short axis view at the level of the chordae tendinae. Left atrial diameter was measured both from right parasternal short and long axis views. Left atrial short

axis and aortic diameter were used to determine Left Atrium/Aorta ratio (LA/Ao). Presence or absence of systolic anterior motion of the mitral valve was also assessed on two-dimensional and M-mode echocardiographic images. Assessment of mitral and pulmonary venous inflow patterns and isovolumetric relaxation time using pulsed-wave spectral Doppler echocardiography was used to assess left ventricular diastolic function. All echoparameters were measured using a concurrent electrocardiogram trace to assist with appropriate timings except where cats were non-compliant.

An overall assessment of the cat was also completed at each visit by both the investigator and the owner to assess whether they felt the cat was very well, well, poor or very poor.

Blood samples were collected at each visit. Biochemistry and haematological variables were measured and urinalysis was performed.

Treatment compliance was assessed at each follow-up visit by counting the number of used and unused tablets and checking the dates and times noted by the owner on the tablet packets.

Safety assessment

The adverse events were reported during the course of the study by the investigators. An adverse event is defined as “any observation in animals that is unfavourable and unintended, and occurs after the use of a veterinary product or investigational veterinary product, whether or not considered to be product related. It is considered serious if fatal, life-threatening or resulting in permanent and prolonged signs in the treated animals”. The adverse events were coded and grouped by organ (System Organ Class) according to the VeDDRA hierarchical structure, as defined by the

European Medicines Agency[f]. Relationship to the study treatment was assessed by the investigator at the time of the exam.

Outcome

The primary endpoint was mortality, defined as death (spontaneous or by euthanasia) due to cardiac causes. The cause of any spontaneous death was evaluated by the investigator.

Secondary efficacy endpoints were morbidity-mortality, defined as the combined incidence of death (spontaneous or by euthanasia) due to cardiac causes and treatment failure as defined by the addition of a forbidden cardiac drug or premature removal due to cardiac causes (e.g. thromboembolic disease).

The safety of spironolactone was assessed by describing the frequency and nature of adverse events and the evolution of haematological, clinical biochemical and urine parameters in both groups. The results of quantitative blood biochemistry variables and quantitative haematological variables recorded at inclusion and at each visit was compared between the two groups.

Statistical methods

Few studies have prospectively evaluated survival in feline heart failure, consequently sample size calculation was not feasible and for the purposes of this pilot study the number of cats to be enrolled was set at 10 animals per group.

The results were analysed with Per Protocol and Intention to Treat populations for the primary endpoint. For the Intention to Treat analysis, any cat having received the tested product or the placebo would be included in the efficacy analysis. Only cats for

which the protocol was strictly respected would be included in the Per Protocol analysis. The significance threshold was set at p=0.05. Analyses were run on commercially available software[g].

The two treatment groups were described and compared on individual criteria on day 0. For qualitative variables chi-square or Fisher's exact test was used according to expected values obtained. In case of normality of the data distribution Student's *t* test was used to compare all continuous variables between the two groups. The variance equality was tested using a Folded F test, in case of unequal variance the Satterthwaite adjustment was used. In case of non-normality, a non-parametric Wilcoxon-Mann-Whitney test was used.

If the end of the follow up period for a case was not related to an endpoint or if the follow up was ongoing at the time of statistical analysis, the cases were censored. Survival curves were generated by the Kaplan-Meier method. Survival analysis was performed using a log rank test to compare the survival of the two treatment groups. The hazard ratio (HR) and its 95% confidence interval (95% CI) were determined based on a univariate analysis using a Cox model. Bivariate Cox proportional hazard analysis was also performed. The global percentages of morbidity-mortality or mortality events at the end of follow-up were compared between groups using Fisher's exact test. Statistical significance was declared at a two-sided p-value of ≤0.05.

Results

Twenty cats were enrolled, 9 in the spironolactone group and 11 in the placebo group, and followed up for a maximum duration of 15 months, unless death or treatment failure occurred. Fifteen cats presented with HCM and 7 cats required hospitalisation. The results of the randomisation is described below in Table 1.

In the spironolactone-treated group, 7 cats (77.8%), had HCM, 1 cat had dilated cardiomyopathy and 1 cat had unclassified cardiomyopathy. In the placebo-treated group 8 cats (72.7%) had HCM, 1 cat had dilated cardiomyopathy, 1 cat had restrictive cardiomyopathy and 1 cat had arrhythmogenic right ventricular cardiomyopathy. There was no significant difference between the two groups in the echocardiographic diagnoses.

Among the 20 cats enrolled, no cat experienced a major deviation such as administration of a forbidden treatment or an evident lack of compliance, therefore, the Per Protocol population was identical to the Intent To Treat population and the safety population and includes all enrolled cases.

Study population at recruitment

The spironolactone and the placebo groups were compared on demographic, clinical, thoracic radiographic, electrocardiographic, echocardiographic and biochemical parameters at inclusion. Baseline characteristics for the two groups are described in Table 2.

Treatment

Cats in the spironolactone-group received a median dose of 2.83 mg/kg (range 2.08

– 3.36 mg/kg, inter-quartile range 0.695), doses administered to individual cats are shown in supplemental Table B on-line. Before inclusion, 17 cats received furosemide with a time averaged daily dose ± standard deviation (SD) of 5.0 (± 2.9) mg/kg and a mean duration before enrolment (± SD) of 11 (± 10) days. Of these, 13 received concomitant benazepril with a time averaged daily dose (± SD) of 0.6 (± 0.4) mg/kg for a mean period (± SD) of 22 (± 53) days.

When included in the study, the time averaged daily dose (± SD) of furosemide for the cats in the spironolactone- and in the placebo-treated groups were respectively 3.0 (± 1.2) mg/kg and 4.8 (± 2.3) mg/kg. For benazepril administration, cats received respectively in the spironolactone- and in the placebo-treated groups 0.4 (± 0.2) mg/kg and 0.6 (± 0.4) mg/kg as a time averaged daily dose (± SD). During the study, 8 cats received a potassium supplementation: 5 in the spironolactone treated group and 3 in the placebo-treated group.

Primary endpoint

In the spironolactone and control groups, respectively, 56% (5/9) and 0% (0/11) of cats completed the 15-month period. With respect to mortality due to cardiac causes, the estimated 15-month survival rate was 78% for the cats treated with spironolactone and conventional therapy, and 0% for the cats in the control group (log rank test, p = 0.011) (Figure 1). The univariate analysis of treatment demonstrated that the spironolactone treated cats had a significant risk reduction for reaching the primary endpoint when compared with the placebo treated cats (HR=0.158; p=0.0226; 95% CI=0.032–0.772).

In the spironolactone group, 22% (2/9) of cats reached the primary endpoint and 82%

(9/11) in the control group (Fisher's exact test, p = 0.0216). Causes of withdrawals not related to cardiac death were worsening of heart failure and a need for forbidden concomitant treatment in 2 of 11 cats (18%) receiving the placebo and death or euthanasia for non-cardiac reasons in 2 of the 9 cats treated with spironolactone (22%).

Because of the significant difference identified between the groups in left atrial size at baseline, this parameter was assessed as a covariate in the Cox model. When atrial size and treatment group were included in a bivariate model, neither had a statistically significant effect on survival, respectively HR=1.53; p=0.53; 95%CI=0.391-6.240 and HR= 0.199; p=0.07; 95%CI=0.026-1.063

Secondary criteria

Morbidity-mortality

Survival analysis showed an estimated 15-month survival rate of 78% in the spironolactone-treated group significantly different to 0% in the placebo-treated group (Log Rank test, p=0.0042). The results of univariate analysis on treatment effects demonstrated a significant risk reduction in the spironolactone treated group as well (HR=0.136; p=0.0119; 95% CI=0.029–0.644).

There is a significant difference in terms of number of events between the two groups (p=0.0005) with morbidity-mortality in the placebo group significantly greater than morbidity-mortality in the spironolactone-treated group (100% versus 22.2%).

All causes of mortality

Survival analysis showed an estimated 15-month survival rate of 56% in the

spironolactone-treated group and 0% in the placebo-treated group, which is not significantly different (Log Rank test, p=0.05). Similarly, the results of the univariate analysis on treatment effects show no significant risk reduction in the spironolactone treated group (HR=0.309; p=0.0604; 95% CI=0.091–1.053). No significant difference between the two groups (p=0.16) with all-cause mortality in terms of number of events (81.8% versus 44.4%) was demonstrated. Cats with HCM represented 75% of the population. For this reason, a post-hoc survival analysis on the sub-population of cats with HCM (n=15, 7 in the spironolactone treated group and 8 in the placebo treated group) was performed and showed an estimated 15-months survival rate of 100% in the spironolactone-treated group, significantly different from 0% in the placebo-treated group (Log Rank test, p=0.0005). There is a significant difference between the two groups (p=0.0014) with mortality due to cardiac causes in the placebo group significantly greater than in the spironolactone-treated group (percentage of events at the end of follow-up: 87.5% versus 0.0%). The univariate analysis of treatment demonstrated that the spironolactone treated cats had a significant risk reduction for reaching the primary endpoint when compared with the placebo treated cats (HR=0.033; p=0.0335; 95% CI=0.001–0.765).

Safety

Adverse events

In total, 39 adverse events were recorded during the study, with 16 recorded in 7 of the cats (78%) in the spironolactone-treated group and 23 recorded in 11 of the cats (100%) in the placebo-treated group. No skin and appendage disorders were recorded during the course of the study. The adverse events according to the system organ

class classification[f] are available in supplemental Table C on-line; most of the events were metabolism and nutrition disorders (mainly hypokalaemia), digestive tract disorders (vomiting) and systemic disorders (loss of appetite, euthanasia or sudden death). Cardiovascular and respiratory tract disorders occurred primarily in the placebo-treated group and were usually a consequence of worsening heart failure.

In the spironolactone-treated group, the investigators assessed 44% of the adverse events as having no relationship to the product and 56% as having a possible or not assessable relationship. In the placebo-treated group, the percentages were respectively 39% and 61%.

Blood and urine parameters

The results for the haematological variables recorded at inclusion and the follow-up visits for the two groups are shown in Supplemental Table D on-line.

The results for the quantitative blood biochemistry variables recorded at inclusion for the two groups are shown in Supplemental table E on-line, the mean ± SD of the results are given. There was a significant difference between the two groups for ALT activity, albumin and potassium. ALT activity and potassium concentration were higher and albumin concentration was lower in the placebo-treated group compared to the spironolactone-treated group. The changes in serum creatinine, urea, potassium and sodium concentrations in the two groups with time are shown in Figure 2.

No abnormalities were diagnosed as a result of urinalysis that required any specific interventions or suggested an adverse drug effect on the urinary system.

Blood pressure

The mean systolic blood pressure recorded at each visit from the cats in the two groups is shown in Supplemental Table F on-line. There was a statistically significant difference between groups in the systolic blood pressure with that of the placebo-treated group being significantly higher at the first visit (p=0.034).

Discussion

This study demonstrated that the addition of spironolactone to conventional cardiac therapy is safe and appears to reduce the risk of cardiac morbidity and mortality in cats with cardiac failure due to cardiomyopathy when compared with conventional therapy alone (ACEi plus furosemide). Of the nine cats treated with spironolactone, five completed the 15 months study compared to none of the 11 cats treated with placebo. These findings are consistent with survival studies on dogs [4] and humans [9, 10, 11] with heart failure, although in this study the primary disease, cardiomyopathy is different from that in the studies on dogs and humans.

However, interpretation of these efficacy data needs to be undertaken with caution due to the small group sizes involved in this study and to the fact that the randomisation process, by chance, led to the inclusion of cats with more severe heart disease (larger left atrial size) in the placebo treated group. The assessment of the impact of left atrial size on the efficacy of spironolactone in this study suggests that this parameter should be included as a stratification factor in future studies.

A previous study in Maine Coon cats with familial hypertrophic cardiomyopathy, spironolactone at 2mg/kg *per os* twice daily for 4 months did not improve the mitral annular velocity nor reduced the left ventricular mass. Four of the 13 cats developed

406 facial dermatitis severe enough to warrant cessation of treatment [14]. Consequently,
407 one of the goals of the present study was to gather safety data on long-term use of
408 spironolactone in a group of out-bred cats to determine whether this problem was as
409 common in the general population. No cases of facial dermatitis were seen in the 9
410 cats treated for up to 15 months in the present study. Furthermore, the prevalence of
411 adverse events reported in the spironolactone treated cats was similar to those
412 reported in the placebo cats suggesting spironolactone is safe to use in cats with
413 naturally occurring heart failure when treated with furosemide and benazepril.
414 Interestingly, hypokalaemia was reported both in the spironolactone-treated group (3
415 cats) and in the placebo-treated group (2 cats). One explanation for the number of
416 sprironolactone-treated cats having hypokalaemia may be that the mean serum
417 potassium concentration was significantly lower in the spironolactone group at
418 baseline when compared to the placebo group. Although these conclusions about the
419 safety of spironolactone are based on a small group of cats exposed to the drug for a
420 long period of time, the data are reassuring and support the design of larger pivotal
421 clinical trials in the future.
422 Most of the cats in this study (15/20) were suffering with HCM. The results of the
423 survival analysis in this population may not therefore be generalizable to cats with
424 other forms of cardiomyopathy. In feline medicine, the classification of myocardial
425 diseases traditionally follows the World Health Organisation definitions [16] and
426 guidelines for making these diagnoses are reported in the literature [8, 17, 18]. In
427 human medicine as well as in veterinary medicine the utility of this traditional
428 classification is starting to be questioned as more information regarding the natural
429 history of these conditions becomes available[h] [19]. There is increasing evidence that

human and feline patients may transit between types of disease as the condition progresses. Different phenotypic expressions of the same condition may exist (possibly related to genetic heterogeneity [20, 21, 22]) and patients with a similar cardiac phenotype may have a very different clinical course[h] [23]. However, the encouraging preliminary results on the HCM population would suggest that only cats with HCM might be enrolled for a future clinical trial, as discussed above, stratified according to left atrial size.

The activation of the RAAS system as a consequence of a fall in cardiac output typically occurs in patients with cardiomyopathy of all types as congestive cardiac failure develops. Consequently there is a degree of commonality in the pathophysiological consequences of the primary disease in patients with cardiomyopathy [8] with increased circulating levels of both aldosterone (initially at least) and angiotensin II demonstrable in patients with cardiac failure. It is likely therefore, that once cardiac failure has developed a similar pathophysiological process is involved in the development of cardiomyopathy and the RAAS has been activated [24].

No significant diuretic effect of spironolactone has been demonstrated in dogs [25, 26] and it may be that the observed clinical benefit from associated with spironolactone use arises primarily from mineralocorticoid blockade effects beyond those of diuresis. It has been shown in rats [27, 28, 29] and human patients [30, 31, 32] that aldosterone induces myocardial and perivascular fibrosis and alters the endothelial function of vessels. Studies performed in human patients with congestive heart failure showed that these effects are counteracted by mineralocorticoid receptor antagonists [32, 33]. Cats with hypertrophic cardiomyopathy have been shown to have significant interstitial

fibrosis and fibrosis of the endocardium [6, 8]. In human medicine there has been a direct correlation shown between severity of diastolic dysfunction and amount of myocardial fibrosis as documented on magnetic resonance imaging scans [34]. It has also been shown in cats that cats with moderate or severe diastolic dysfunction have a poorer prognosis [35], it is likely that this effect, at least in part is due to myocardial fibrosis. Mineralocorticoid receptor antagonists like spironolactone have been shown to inhibit aldosterone-induced myocardial fibrosis [5] and to reduce remodelling of the vascular smooth muscle cells and myocytes [3]. This action may therefore represent an additional benefit to using these agents in feline cardiomyopathy where inhibition of profibrotic and prohypertrophic neurohormones is a reasonable treatment goal in cardiomyopathies and other cardiac diseases [36]. More recently it has been suggested that the benefit of mineralocorticoid receptor antagonists now extends to the early phases of myocardial damage [37] and to heart failure with preserved ejection fraction, a situation which could describe many cats with cardiomyopathy [38]. Other recent work also suggests that the effects of angiotensin II and mineralocorticoid receptor activation in the heart are additive [39]. This observation may be relevant to the clinical use of ACEi and mineralocorticoid receptor antagonists in combination in heart failure.

The study has a number of limitations, which are largely a consequence of the fact that it was a small scale pilot study. No power calculation was done to establish sample sizes and the number of patients in each group is small. There were differences, some significant, between the treatment group and the placebo group in some of the baseline variables. In particular, in the placebo group the body weight at inclusion was lower than that of the cats in the treatment group and the body condition score of

placebo-treated cats was significantly lower than that of the cats in the treatment group. Similarly, in the placebo group there was a significantly higher prevalence of other abnormalities on the electrocardiogram at presentation. In addition, on echocardiographic assessment at inclusion into the study there was a significant difference between the two groups in the LA/Ao ratio. Any future study might include stratification of cats at inclusion according to severity of heart failure as assessed by left atrial size or biomarker levels [40]. Another limitation is the fact that no dose determination study was conducted prior to this pilot study. However, in practice, veterinarians have been using spironolactone for several years using a dose range of 2 to 4 mg/kg [12, 13] and the allometric approach used in this study provided a similar dose range (1.72-3.33 mg/kg). The study does suggest that spironolactone is safe to use in cats and provides data which would permit better case selection and stratification and a power calculation to be done for a full clinical trial.

Conclusion

Spironolactone therapy over a 15-month period in cats with heart failure secondary to cardiomyopathy was safe to use and demonstrated a potentially beneficial effect when added to conventional therapy. This finding needs to be confirmed by a large scale clinical trial, with stratification at inclusion according to parameters which have recently been demonstrated to be linked to prognosis.

Conflict of Interest Declarations:

The study was funded by Ceva Sante Animale. Emilie Guillot, Catherine Garelli-Paar and Vanessa Grassi are employees of Ceva Sante Animale. Rachel James and Malcom Cobb have received funding from Ceva Sante Animale within the last 5 years for some or all of the following activities: research, travel, speaking fees and preparation of educational materials.

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Figure captions

Figure 1. Kaplan-Meier survival curves, showing the number of patients surviving within the populations treated with spironolactone and placebo at different time points.

Figure 2. Serum creatinine, urea, potassium and sodium mean concentrations for the treatment and placebo groups with time, showing changes in these parameters over the course of the study.

V = visit number

Table 1. Randomisation of the enrolled cases into the treatment and placebo groups according to both stratifications					
	Cats with hypertrophic cardiomyopathy		**Cats with other type of cardiomyopathy**		**Total**
Need for hospitalisation	***Spironolactone***	***Placebo***	***Spironolactone***	***Placebo***	
Yes	2	3	1	1	7
No	5	5	1	2	13
TOTAL	7	8	2	3	20

Table 2. Baseline characteristics of the cats in the treatment and placebo groups at enrolment (mean ± standard deviation or number and percentage)				
		Spironolactone	**Placebo**	**p-value**
Clinical parameters				
	Weight (kg)	4.2 ± 1.3 (9)	3.6 ± 0.9 (11)	0.22
	Age (years)	7.0 ± 4.9 (9)	10.3 ± 3.5 (11)	0.09
	Breed - Domestic Short Hair - Ragdoll - Birman - Siamese	 8 - - 1	 9 1 1 -	
	Blood pressure (mm Hg)	115± 22	137 ± 20	**0.034**
	Body Condition Score	4.3 ± 1.0	3.3 ± 1.2	**0.048**
Electrocardiography				
	Heart rate	185± 28	185±38	0.97
	Normal sinus rhythm	8/9 (89%)	7/11 (64%)	0.32
	Ventricular premature complexes	4/9 (44%)	3/11 (27%)	0.64
	Ventricular tachycardia	1/9 (11%)	0/11 (0%)	0.45
	Supraventricular premature complexes	0/9 (0%)	2/11 (18%)	0.48
	Atrial fibrillation	0/9 (0%)	2/11 (18%)	0.48
	Other arrythmia	0/9 (0%)	1/11 (9%)	1.00
	Other abnormality*	0/9 (0%)	7/11 (64%)	**0.0047**
Echocardiography				
	Interventricular septum thickness in diastole (mm)	5.6± 1.5 (9)	6.2±2.1(11)	0.48
	LV internal dimension in diastole (mm)	14.5± 4.0 (9)	15.1±2.4(11)	0.72

	LV free wall thickness in diastole (mm)	6.0± 2.3 (9)	5.7±1.5 (11)	0.68
	Interventricular septum thickness in systole (mm)	7.7± 1.7 (9)	7.7±2.1 (11)	0.97
	LV internal dimension in systole (mm)	7.8± 3.6 (9)	9.9±3.4 (11)	0.20
	LV free wall thickness in systole (mm)	8.2± 2.5 (9)	7.2±2.0 (11)	0.30
	LV shortening fraction (mm)	45.0± 14.9 (9)	35.4±14.1 (11)	0.15
	Left Atrium short axis (mm)	17.0± 2.6 (9)	20.0±4.1 (11)	0.077
	Left Atrium diameter from right parasternal long axis view (mm)	18.5± 2.4 (9)	22.6±5.0 (11)	0.051
	Aorta diameter (mm)	9.0± 0.8 (9)	8.0±0.6 (11)	**0.006**
	Left atrium/Aorta	1.9± 0.3 (9)	2.5±0.6 (11)	**0.013**

*Right bundle branch block, negative QRS in lead I but positive in leads II and III, tall and wide P waves, right axis deviation, second degree atrioventricular block, ST elevation, left bundle branch block.

Supplemental Table A. Visit schedule

V: visit number; D: day number; W: week number; M: month number

Visit No.	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**
Day No. (Week No.) (Month No.)	D0	D7±1 (W 1)	D28±2 (W 4) (M1)	D56±2 (W 8) (M2)	D84±3 (W 12) (M3)	D140±3 (W 20) (M5)	D210±3 (W 30) (M7)	D280±3 (W 40) (M10)	D420±3 (W 60) (M15)
Clinical examination	X	X	X	X	X	X	X	X	X
Questionnaire	X	X	X	X	X	X	X	X	X
Haematology	X				X			X	X
Biochemistry	X	X	X	X	X	X	X	X	X
Packed cell volume	X	X	X	X	X	X	X	X	X
Thyroid hormone	X								
Urine	X		X		X	X		X	X
Pharmacokinetic sample	X	X	X	X	X	X	X	X	X
Electrocardiogram	X				X			X	X
Blood Pressure	X	X	X	X	X	X	X	X	X
Echocardiography	X				X			X	X
Thoracic Radiographs	X							X	X

Supplemental Table B. Doses of spironolactone administered respectively to each cat in the spironolactone-group	
Cat	***Spironolactone dose (mg/kg)***
Cat 01 - A	3.26
Cat 02 - A	2.08
Cat 03 - A	2.90
Cat 04 - A	2.33
Cat 05 - A	2.83
Cat 06 - A	2.63
Cat 07 - A	3.03
Cat 08 - A	2.44
Cat 09 - A	3.13

Supplemental Table C. Number of adverse events in the treatment and placebo groups.						
System Organ Class	Number of events reported			Number of cats presenting with the event (at least once)		
	Total	Spironolactone	Placebo	Total	Spironolactone	Placebo
Cardio-vascular system	8	1	7	8	1	7
Digestive tract	3	3	0	2	2	0
Ear and labyrinth	1	1	0	1	1	0
Hepato-biliary	1	1	0	1	1	0
Metabolism and nutrition	8	6	2	5	3	2
Renal and urinary	1	0	1	1	0	1
Respiratory tract	9	1	8	5	1	4
Systemic	8	3	5	6	2	4
Total	**39**	**16**	**23**	**18**	**7**	**11**

Supplemental Table D. Range of haematological variables (number of cats) for the cats in the treatment and placebo groups at each visit										
	Haematocrit (%)		**Packed cell volume (l/L)**		**Total platelet count ($X10^{12}$/L)**		**Total Red cell count ($X10^{9}$/L)**		**Total white cell count ($X10^{9}$/L)**	
Time	**Spironolactone**	**Placebo**	**Spironolactone**	**Placebo**	**Spironolactone**	**Placebo**	**Spironolactone**	**Placebo**	**Spironolactone**	**Placebo**
V1	31.8 – 44.3 (5)	23.0 – 42.5 (9)	32.0 (1)	27.0 – 42.0 (2)	75 - 757 (5)	65 - 582 (9)	6.1 – 9.7 (5)	4.4 – 9.6 (9)	5.2 – 14.7 (5)	5.5 – 32.1 (9)
V5	12.8 – 39.4 (4)	22.9 – 39.3 (3)	30.0 (1)	36.0 (1)	131 - 1046 (4)	297 - 378 (3)	3.0 – 9.5 (4)	5.4 – 9.6 (3)	5.3 – 10.1 (4)	6.3 – 14.9 (3)
V8	29.7 – 34.4 (3)	(0)	28.0-32.0 (4)	(0)	219 - 703 (3)	(0)	7.1 – 9.2 (4)	(0)	4.4 – 18.6 (4)	(0)
V9	26.4 – 30.0 (2)	(0)	32.0 – 35.0 (2)	(0)	310 - 1073 (3)	(0)	5.7 – 7.0 (3)	(0)	3.4 – 11.9 (3)	(0)

Supplemental Table E. Comparison of the baseline clinical biochemistry parameters of the cats in the treatment and placebo groups at enrolment (mean ± SD)				
		Spironolactone	**Placebo**	**p-value**
	Alkaline phosphatase (U/l)	31.7±14.1	44.2±34.4	0.41
	Alanine aminotransferase (U/l)	47.0±19.9	89.2±52.3	**0.040**
	Albumin (g/l)	32.7±1.6	29.3±3.5	**0.044**
	Amylase (U/l)	950±281	1086±304	0.43
	Chloride (U/l)	114±5	119±4	0.08
	Cholesterol (mmol/l)	3.9±1.1	4.8±1.2	0.16
	Creatinine (µmol/l)	154±28	189±56	0.15
	Globulin (g/l)	45.0±5.8	47.1±11.4	0.68
	Phosphate (mmol/l)	1.6±0.2	1.5±0.2	0.46
	Potassium (mmol/l)	3.3±0.2	4.1±0.7	**0.028**
	Sodium (mmol/l)	155±8	160±6	0.25
	Bilirubin (µmol/l)	5.8±4.0	5.6±1.5	0.87
	Calcium (mmol/l)	2.4±0.1	2.4±0.2	0.88
	Protein (g/l)	78±6	76±12	0.80
	Urea (mmol/l)	11.6±7.9	14.5±7.5	0.41

Supplemental Table F. Systolic blood pressure (median and range) in mmHg (number of cats) in the treatment and placebo groups at each visit (V: visit number)									
	V1	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**
Spironolactone	120 85 – 144 (9)	121 106 – 134 (8)	128 120-148 (7)	132 104 – 141 (7)	132 112 – 144 (7)	136 120 – 158 (5)	134 96 – 151 (6)	116 112 – 152 (5)	124 120-128 (5)
Placebo	140 100 – 168 (11)	150 108 – 166 (11)	129 108 – 200 (10)	138 124 – 176 (6)	142 118 – 180 (5)	142 108 – 148 (3)	112 110 – 114 (2)		

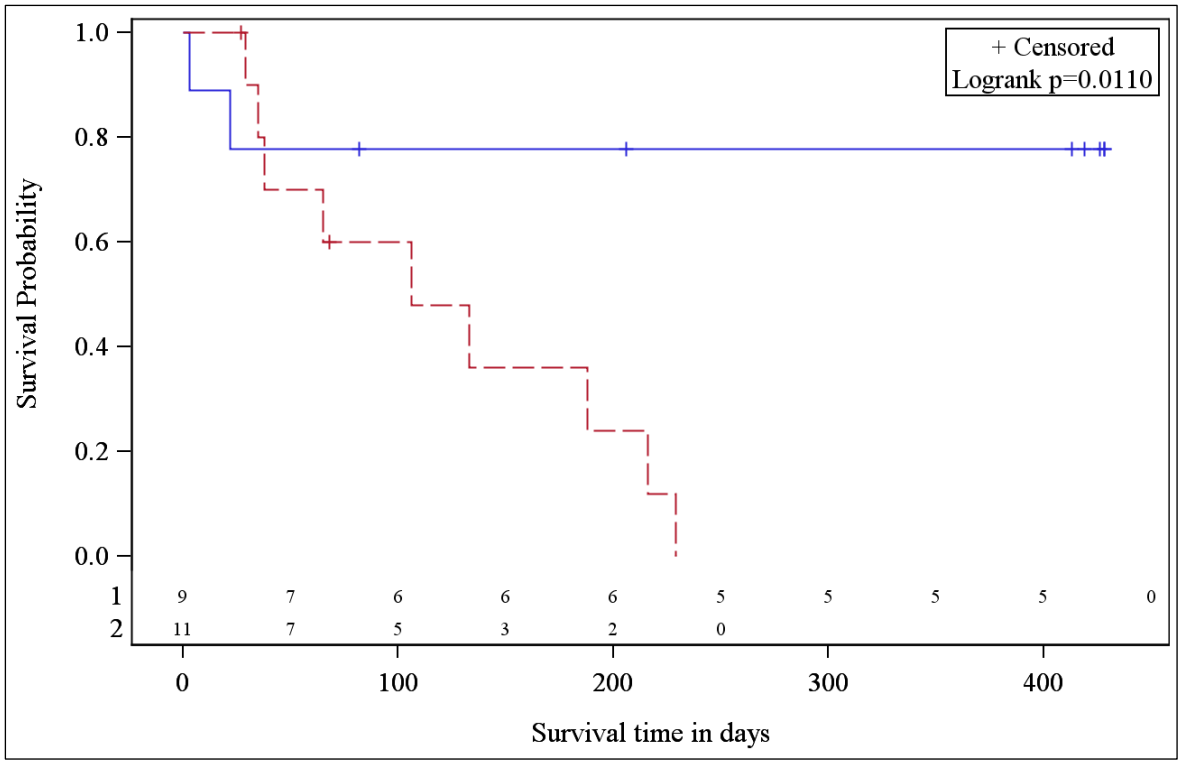
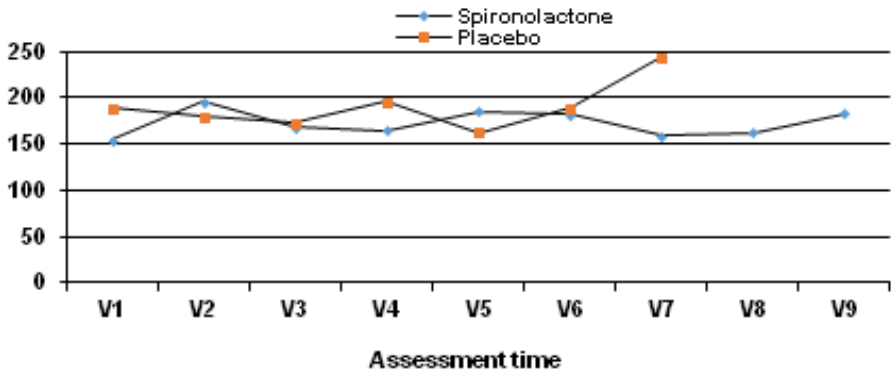
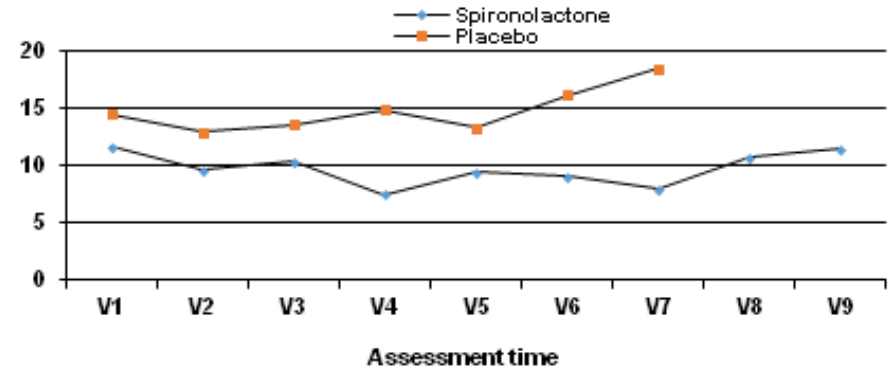
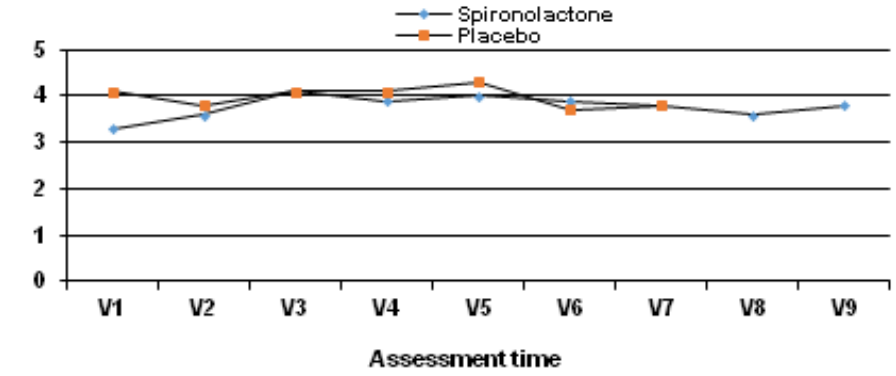

+ Censored
Logrank p=0.0110
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
1 9 7 6 6 6 5 5 5 5 0
2 11 7 5 3 2 0
0
100
200
300
400
Survival time in days
Group 1: Spironolactone ----------
Group 2: Placebo ----------

Figure 1

Creatinine (µmol/l)	
Urea (mmol/l)	
Potassium (mmol/l)	

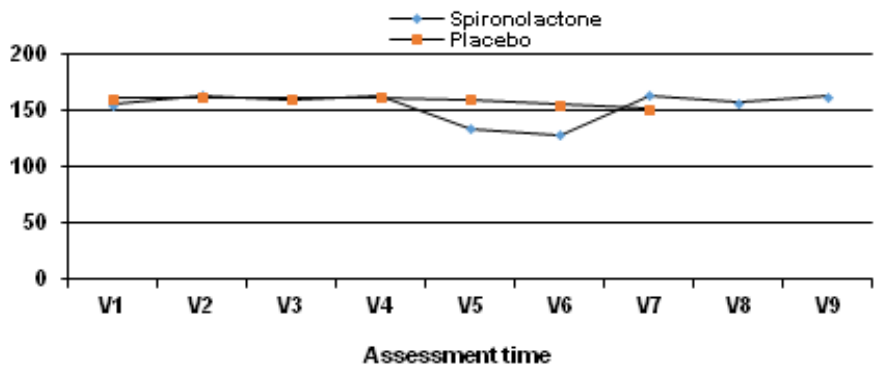
Sodium (mmol/l)	

Figure 2